Small Brain-on-a-Chip Promises Big Payoffs

Livermore's new investigational platform could provide an innovative means for better understanding brain pathology and for developing countermeasures to chemical and biological warfare agents.

IN a telling example of Lawrence Livermore's pioneering marriage of biology and engineering, Laboratory researchers have developed a "brain-on-a-chip"—the newest embodiment of an integrated system designed to accurately evaluate the effects of potentially harmful chemicals, viruses, and drugs on humans without relying on animal or human test subjects. In conjunction with an artificial blood–brain barrier (BBB), the device simulates the central nervous system (CNS) by recording activity from multiple brain cell types deposited and grown onto a small platform embedded with microelectrode arrays.

The brain-on-a-chip holds significant promise for national security and broader applications. For example, the device could be used for determining how soldiers are affected by exposure to chemical and biological weapons and the effectiveness of potential countermeasures and prophylactic pretreatments. The technology may also offer a breakthrough means to more quickly predict the effects on the brain from candidate drugs developed to treat neurological disorders. Finally, it could help scientists understand how brain cells function, connect, and interact to combat neurological impairments and illnesses such as Parkinson's disease and epilepsy.

The device is part of the Laboratory's iCHIP (in vitro chip-based human investigational platform) project—a broad initiative at Livermore to advance human health with a focus on understanding, diagnosing, and potentially treating human neural problems and diseases. (See *S&TR*, March 2014, pp. 16–19.) The research effort applies Livermore core capabilities in bioscience, bioengineering, materials science, and high-performance computing as well as expertise in forensic science and microfabrication. Developed through an accomplished multidisciplinary team, which this year included 11 scientists and engineers, 5 postdoctoral researchers, and

5 summer students, iCHIP technologies are becoming a faster, less expensive, and more effective method for evaluating the body's response to human-made and various natural threats.

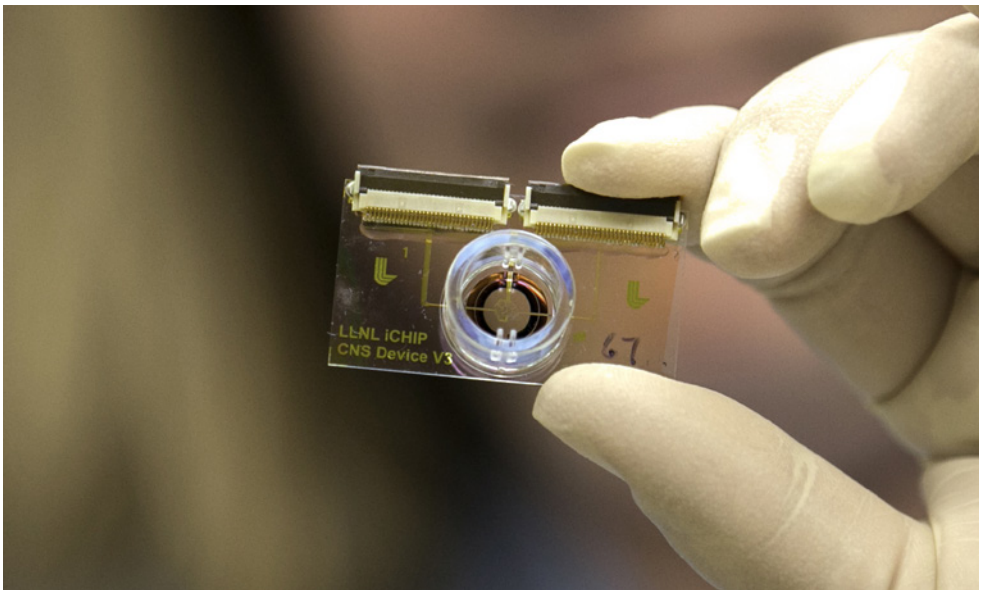
Initiatives Prove Fruitful

The Laboratory's Center for Micro and Nanotechnology is a dedicated fabrication and prototyping facility with extensive experience manufacturing biocompatible microelectrode arrays for recording (and optionally, generating) neural signals. Fabricated at the center's Biomedical Foundry, the arrays gained national attention as part of the first commercialized artificial retina, for which the Laboratory played an important development role. (See *S&TR*, October/November 2009, pp. 14–15.)

Since that time, the Laboratory Directed Research and Development (LDRD) Program has supported iCHIP projects by funding two Strategic Initiatives (SIs). This type of research investment aims to answer key science, technology, and engineering challenges. The first SI, which ended in 2015, allowed the research team to integrate the biocompatible microelectrode array technology into four separate organ-on-a-chip devices: CNS, BBB, peripheral nervous system (PNS), and heart (see the box on p. 10). The second SI, which will end in late 2019, focuses on further developing CNS and associated BBB platforms. According to biologist Kris Kulp, deputy division leader for Livermore's Biosciences and Biotechnology Division, "The first SI confirmed we could build biocompatible engineered systems that support a healthy culture of different cell types." With the platform built and validated, the aim in the second SI, says Kulp, is to discover "what kind of pressing biological questions can be answered with our engineered platform, especially with regard to examining the brain's response to chemical warfare agents."

Engineer Elizabeth Wheeler, a principal investigator (PI) for the first SI and a co-PI for the second one, notes that U.S. warfighters as well as civilian populations face a threat from exposure to chemical warfare agents. Although banned by international treaty, chemical weapons have been used sporadically in Mideast conflicts. Following exposure, these compounds quickly affect CNS, PNS, and other organs and can cause seizures, paralysis, and death. To streamline countermeasure development, researchers need an experimental model that produces more human-relevant data and measurements than do current assays.

According to biologist and co-PI Nick Fischer, a reliable experimental model must mirror human brain function. This type of model can be accomplished by carefully integrating key physiological parameters, such as a three-dimensional (3D) architecture, implementing human neurons, and incorporating neuronal "support" cells. The current brain-on-a-chip platform fulfills all of these requirements and is further complemented by a BBB component. In this way, the device offers the promise of more rapidly developing new antidotes to chemical (and biological) warfare agents without depending on unreliable animal testing. Indeed, more than 90 percent of candidate pharmaceuticals that pass animal studies fail in human trials. In addition, although simple human cell cultures provide basic insight, they are often too far removed from the complexities of the entire nervous system to accurately mirror the responses of the brain.



Livermore's iCHIP (in vitro chip-based human investigational platform) is a miniature external replication of a human organ, integrating biology and engineering with a combination of microfluidics and multielectrode arrays. The iCHIP team has developed platforms for four separate organs: central nervous system (CNS, shown here), blood–brain barrier, peripheral nervous system, and heart. (Photo by Julie Russell.)

Mimicking the Brain

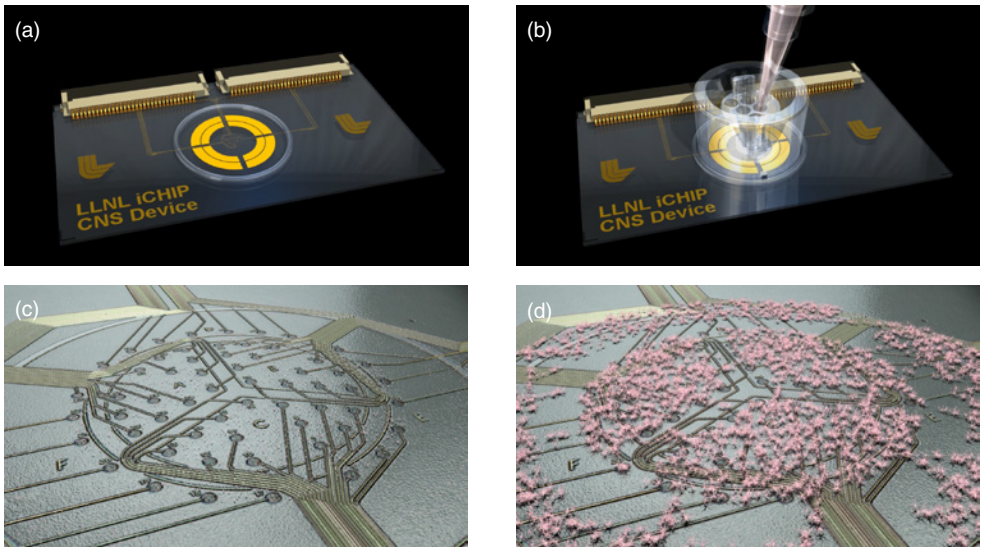
A key to the brain-on-a-chip is Livermore's ability to tailor the design and fabrication of the microelectrode arrays, which capture the patterns of neural cells' action potentials—the "bursts" or spikes of electrical energy that cells emit when communicating with each other. "The microelectrodes serve as 'microphones' that listen to the neurons," says biologist Heather Enright. The electrical signals are recorded from cells that are positioned on or near an electrode. The arrays

record changes in the neural cells' electrophysiology (electrical activity) and viability in response to chemical exposures.

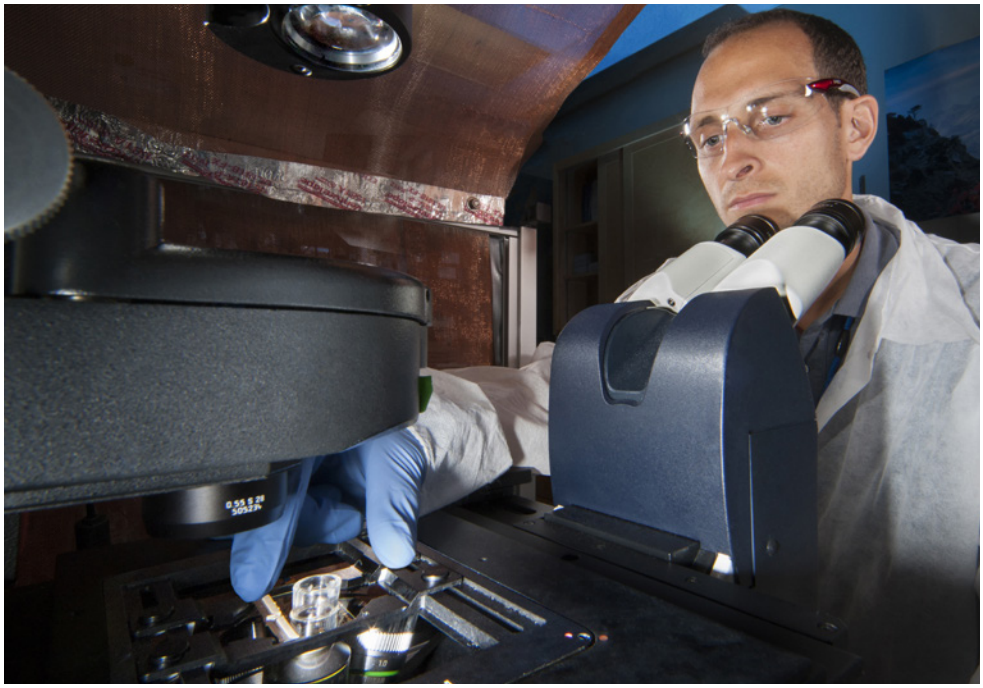
Cells are grown inside experimental wells that sit on top of the microelectrode arrays embedded in the platform. The platforms are designed to promote cell health and longevity, and their surfaces support cell growth and adherence. The cells' viability can be confirmed both optically by brightfield and fluorescent microscopy and electrophysiologically by the embedded microelectrodes. After 10 to 14 days, the microelectrodes start to pick up electrical signals, and by 21 days the cells form a functioning communication network.

As part of the first SI, the team built a CNS-based platform that simultaneously cultured rat brain cells from the cortex (the brain's outer layer of neurons) and hippocampus seeded in different sections at the bottom of the platform's well. To recreate different regions of the human brain, researchers positioned the cells on the platform based on their relative orientation in vivo.

Engineer David Soscia led a team that developed a microfabricated, funnel-like insert made for any type of chip platform or cell type. It enables precise placement of different cell populations onto smaller areas within the well and in closer proximity to neighboring populations. Cells are added to the insert with a micropipette and then settle via gravity flow through the insert for precise deposition onto the microelectrode array. Once the cells are deposited, the insert is removed and the cells become established, sending out long processes (axons and dendrites) to communicate with each other. The lack of physical barriers or chemically treated surfaces is unique to Livermore's "multiregion" CNS platform. Since no physical barriers exist, the hippocampal and cortical neurons can freely communicate not only with themselves, but with each other. Importantly, both cell types retain their



(a) Lawrence Livermore's brain-on-a-chip is designed to promote the health and longevity of multiple cell types. (b) A microfabricated, funnel-like insert enables precise placement of different cell populations onto small areas within a protruding "well." Cells are added to the insert with a micropipette and then deposit onto the (c) microelectrode array at the center of the platform. (d) The insert is removed, and the cells become established and begin to communicate with each other. (Renderings by Kwei-Yu Chu.)



Engineer David Soscia uses a microscope to examine cells established within the brain-on-a-chip. (Photo by Randy Wong.)

signature shape, viability, and function, despite being co-located.

The researchers analyzed the rodent brain cells' electrical activity for up to 30 days and found that the cells on the platform displayed the same physiological responses previously described by researchers using live animals. The Livermore team also showed that some features of hippocampal cells' electrical activity, such as the spiking rate, were significantly higher when cultured together with cells obtained from the cortex.

From Rodents to Humans

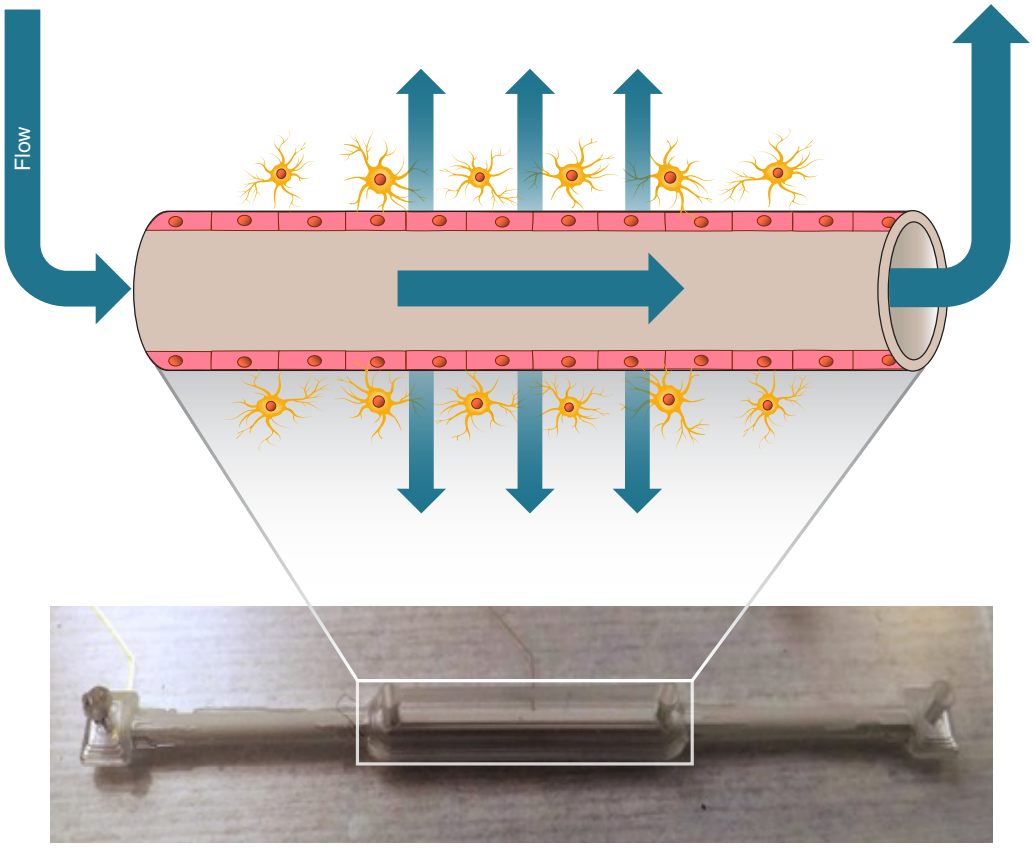
During the second SI, the team began building on their initial results and worked to more closely mimic the brain's two-dimensional cellular microenvironment by increasing the complexity of the cell culture to include neural support cells (astrocytes and oligodendrocytes). In collaboration with Stanford University, they also began experiments with human cells. Fischer explains that the human brain includes distinct but interconnected regions of neurons and supporting cells, and any accurate model must reflect that heterogeneity. Despite this requirement, most in vitro research platforms have focused on populations of a single type of cell or even individual human cells.

In the mixed-cell platform, astrocytes provide structural support, secrete growth factors, and modulate electrical transmissions. Oligodendrocytes produce the myelin that insulates neurons' axons, which carry electrical impulses away from the cell body. As expected, the cultures of neurons combined with astrocytes and oligodendrocytes to form robust neuronal networks exhibiting greater synchronized activity than simple cultures of neurons alone.

The researchers launched a significant effort to validate the technology and demonstrate that the cells on the engineered platforms could generate human-relevant data. They evaluated cell response to chemical agents, surrogates, and well-documented compounds known to affect CNS by either exciting or suppressing electrical activity. The work included use of surrogate chemical agents from biological laboratories at Lawrence Livermore as well as real chemical agents that are strictly controlled at the site's Forensic Science Center—one of two U.S. laboratories with international certification to handle chemical warfare agents.

The validation effort included a direct comparison between cultured rodent neurons and live animal models. An electrode array called the Livermore Flexible Probe (see *S&TR*, June 2018, pp. 4–11) was implanted into a rat's cortex by former Livermore engineer Anna Belle, who co-led the study. In parallel, rat cortical neurons were cultured with the brain-on-a-chip. Both in vivo and in vitro cells were exposed to various chemicals. In the case of an anesthetic dose of ketamine, both cell types showed repressed neural activity, as expected. However, cells on the in vitro platform did not completely mirror the response of the animal, likely resulting from complexities such as metabolic breakdown that are not captured in an isolated in vitro system. The team also tested the chemical atropine (a treatment for nerve agent exposure) and found that the results of the in vitro experiments were similar to those using in vivo cells.

Enright notes that although testing with rodent models and extrapolating the results to humans is not ideal, the ability to compare neuron responses in live animals to those in rodent brain-on-a-chip cells holds considerable value. Tests can also help determine the difference



The blood–brain barrier platform incorporates flow along and across a hollow fiber, which is coated with cultured human endothelial cells (pink rectangles) on the inside and astrocytes (yellow figures) on the outside.

between the responses of human and rodent brain cells cultured on Livermore platforms. By correlating data between both live animals and cell cultures and human and rodent cells, the team can more confidently predict where the platform may be most effective in studies to develop countermeasures.

The team is now working to evaluate complex, 3D neuronal cultures that allow noninvasive interrogation with microelectrodes. "Seeding human neurons in 3D gives us a more realistic morphology, especially when including support cells," says Fischer. "Neurons and other brain cells behave differently when in a 3D environment. Their firing patterns differ, including more synchronized bursts of electrical activity." Soscia leads the effort to develop the prototype 3D configuration in which microelectrode arrays are located vertically along biocompatible polymer pillars. Neuronal cells are seeded around the pillars in a biocompatible hydrogel matrix to provide structural support (an effort spearheaded by postdoctoral researcher Doris Lam). "We want a cell culture depth of approximately 500 micrometers compared to the 20-micrometer depth we have obtained thus far," says Fischer.

A Highly Selective Organ

Developing an artificial BBB model is an important complement to the brain-on-a-chip platform. Indeed, understanding which chemicals cross this highly regulated barrier has significant implications on their ultimate effect on the in vivo CNS. "The blood–brain barrier is the brain's first line of defense," comments biomedical engineer Monica Moya, who leads development of the specialized platform. "It decides what substances are allowed to pass through into the brain." For a drug (or toxin) to affect CNS, it must pass through the barrier. Conversely, BBB breakdown is involved in brain pathology and toxicology.

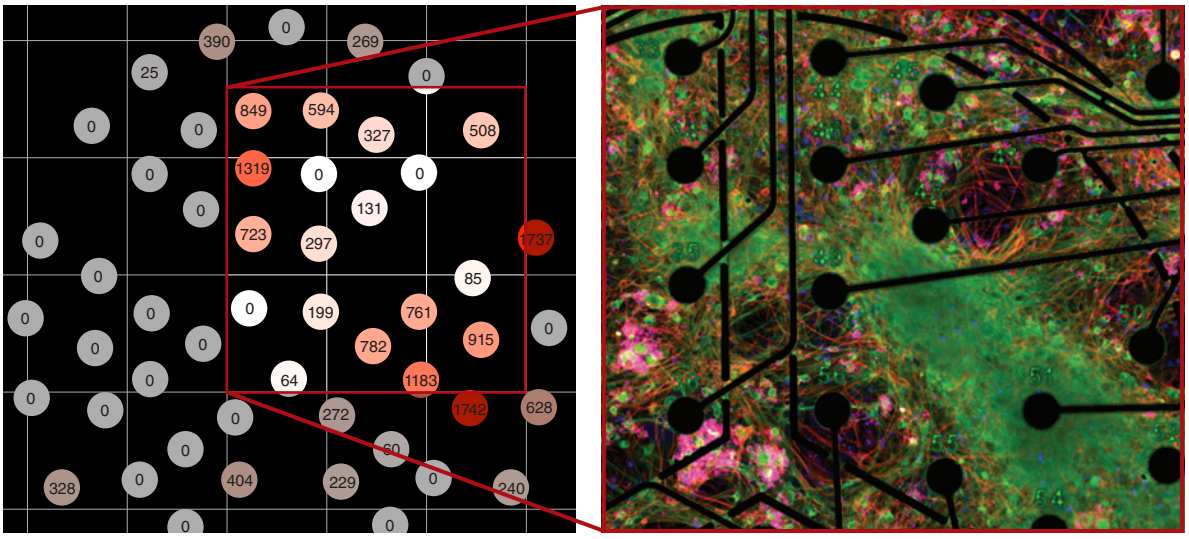
A critical task for the second SI has been to optimize and validate the BBB device. Human endothelial cells (that line blood vessels) are cultured on the inside of porous hollow fibers until a tight monolayer of cells forms, and astrocytes are cultured on the outside of the fibers as support cells. Fluid that contains nutrients continuously flows through the cell-coated fibers. Compounds travel through tight junctions between endothelial cells or through the cells themselves, while pumps rhythmically pass fluid along the cell monolayer, causing them to experience shear stress similar to what the in vivo BBB sustains.

Data from the device were validated by measuring the cell response to chemical compounds that are known to cross, disrupt, or be blocked by the barrier. Researchers used a variety of biological molecules to measure BBB function and permeability and demonstrated that key barrier features, for example drug efflux pumps, were active in the engineered device. Exposure to histamine and a cell-signaling protein increased permeability in vivo, and both chemicals disrupted the barrier's integrity, as expected.

Making Sense of Data

An essential element of the second SI is performing data analytics and computational modeling to help scientists better understand neuronal networks and how cells communicate. Computational models serve as a tool for predicting the effects of compounds on the brain and speeding development of therapeutic regimens for exposure to chemical agents.

Data scientist Ana Paula De Oliveira Sales and postdoctoral researcher Jose Cadena Pico are analyzing the data collected by the microelectrodes to study how neurons' electrophysiology changes over time and in response to environmental conditions and compounds. "Modeling the brain cell networks helps us make sense of all the data," says De Oliveira



(far left) Scientists map electrical activity hotspots (shades of red) to cell composition. (inset) The information is used to better understand communication between brain cells and cell types, as recorded by electrodes (black circles).

Platform Enables Study of Different Biological Systems

Lawrence Livermore's iCHIP (in vitro chip-based human investigational platform) holds promise as a tool for speeding development of medical countermeasures for biosecurity applications and improving the overall drug discovery process. iCHIP devices combine human cells, tissue engineering, and microfluidics to reproduce the body's physiological response under an array of conditions. In a project funded through a Laboratory Directed Research and Development Program Strategic Initiative, Livermore researchers have integrated the biocompatible microelectrode array produced in-house into four separate organ-on-a-chip platforms, including ones for the heart and peripheral nervous system (PNS).

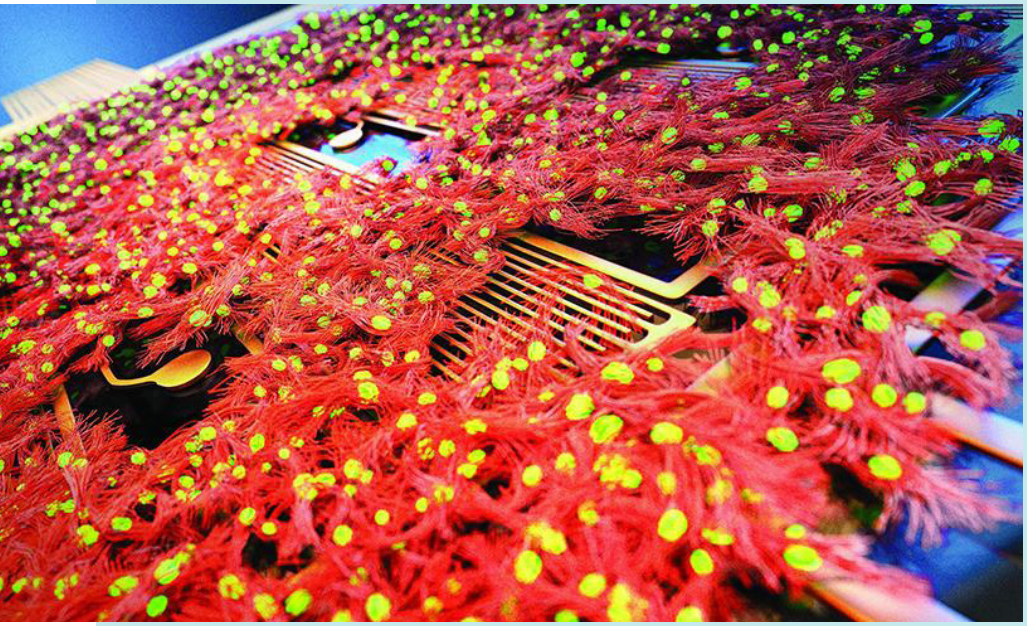
Livermore's heart-on-a-chip, developed by materials scientist Fang Qian, offers a noninvasive method for measuring the adhesion, health, and contractility of heart cells simultaneously in real time—a first in cardiac research. The platform's heart cells naturally grow into two-dimensional tissue that starts to beat after just two days in culture. One of two independent microelectrode arrays integrated into the device monitors the electrical activity of the cells, while the second array measures impedance, which correlates with contraction. In studies, when the cells were exposed to norepinephrine, a stimulant drug used to treat low blood pressure, both the electrical signal and firing rate increased, as happens in human hearts. In contrast, when researchers applied blebbistatin, an excitation–contraction decoupling compound, the cells stopped beating although the electrical signals continued, as expected.

Cardiotoxicity—damage to the heart cells—is a major cause of why promising drug candidates fail. The heart-on-a-chip could help assess the effects of candidate pharmaceuticals on heart tissue much earlier in the drug discovery process. Such a device would also decrease the time needed for new drug trials and ensure potentially lifesaving drugs are safe and effective while reducing the need for human and animal testing.

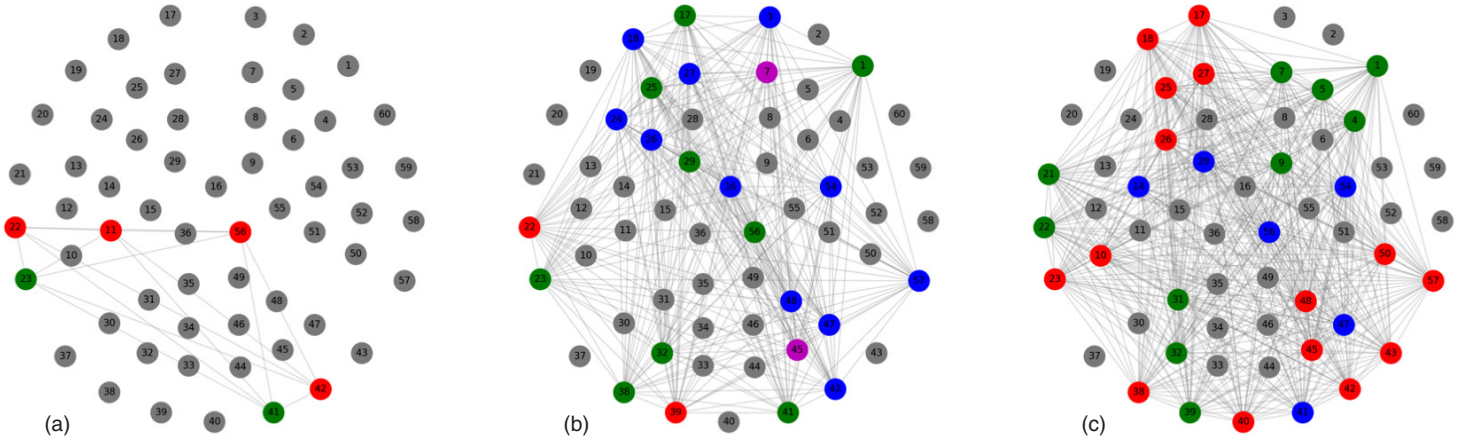
The effects of new drugs and toxins on PNS tissues, which connect the central nervous system to organs and limbs, are often investigated using neurons isolated from human dorsal root ganglia (DRG). Located along the spinal nerves, DRG are the cell bodies of sensory neurons, which have long axons (extensions) that are activated by pressure, temperature, and chemical stimuli. DRGs are also important for processing both acute and chronic pain. They thus serve as an excellent model for studying the neurotoxic effects of chemicals in the body.

PNS tissues were the first biological system components incorporated into an iCHIP. Biologist Kris Kulp says that previous studies using DRG neurons described changes in electrophysiology proceeding from variations in culture conditions and exposure to viral proteins, drugs, and chemicals. However, these studies used the traditional "patch-clamp" technique, which is used for investigating the electrophysiology of single neurons. As a result, the data obtained were limited to tens of single cells, making statistically relevant data impractical. In addition, the patch-clamp method disrupts the neuron's membrane, killing the cell and making chronic chemical testing impossible.

The PNS-on-a-chip device offers strong advantages over other models because it records results from hundreds of DRG neurons for long-term testing and evaluation of chemical and toxic effects. The team seeded human DRG neurons onto the platform and recorded the patterns of the tissues' action potentials with the embedded microelectrode array. The researchers also integrated pH sensors to indicate cell activity, metabolism, and general health. The cells were cultured for several weeks and tested for their response to various chemicals, including capsaicin (a compound found in chili peppers that triggers neural pain response), adenosine tri-phosphate (activates neuron receptors), and potassium chloride (causes neural membrane depolarization). Results of the tests were consistent with previously documented human-derived data.



The heart-on-a-chip measures the effects of various compounds on human heart cells. (Rendering by Ryan Chen.)



An array of 60 electrodes is either inactive (grey) or active and part of a specific community (shown in different colors) after (a) 16, (b) 23, and (c) 30 days in culture.

Sales. "Sometimes a neuron will emit a spike, which indicates that a message is being passed to one or several cells." The researchers' computational model attempts to illustrate how network activity can reflect the complexity of the culture and how this process evolves. "We are looking at cell communities that form and the chatter that goes on within them as well as communication with neighboring communities," she says.

De Oliveira Sales notes that the Laboratory has significant capability to examine the brain-on-a-chip cells using brightfield microscopy. Such analysis can reveal whether a cell is solitary, clumped with others, or appears healthy or diseased. The computational team is also developing methods to autonomously correlate appearance and apparent health of the cells with their recorded electrical activity.

Many Potential Benefits

Livermore researchers are showing that their iCHIP devices provide higher quality, more reliable human-relevant data than other investigative models. Enright says, "We have made considerable progress over the past few years. The brain-on-a-chip provides a unique capability."

By recreating the microenvironment and function of brain tissues, the brain-on-a-chip allows the study of how cells form networks, how they communicate, and how that communication changes when cells are combined with, or located close to, a different cell type. The platform will also allow researchers to analyze how disease spreads through the brain and more accurately model epilepsy and other debilitating conditions. For example, the platform could aid the study of human seizure response—a reaction caused by some chemical warfare agents—to help physicians better understand how to treat the condition.

For protecting the warfighter, the device may greatly advance development of effective countermeasures for exposure to chemical (and biological) agents. For example, researchers could potentially predict how warfighters are likely to be affected by long-term, low-level exposure to chemical agents. The brain-on-a-chip may also help scientists determine if certain types of neurons are more susceptible to these toxins than others. Scientists could also screen compounds for prophylactic use before entering at-risk environments. Existing pretreatments for U.S. warfighters have unpleasant side effects. Therefore, development of alternatives that are equally effective but better tolerated is of significant interest.

Conventional development of new pharmaceuticals and antidotes to toxic compounds currently takes years, costs billions of dollars, and relies extensively on animal testing, which can lead to inaccurate predictions about the likely human response. Federal agencies need a faster, cheaper, more flexible method for addressing threats. The Livermore team has discussed follow-on funding with representatives from the National Institutes of Health and the Department of Defense. Fischer notes the brain-on-a-chip effort dovetails with Lawrence Livermore's overall mission of national and global security. In a few years, U.S. warfighters—as well as the general public—may have an improved tool for thwarting agents of chemical warfare and, quite possibly, agents of human disease.

—Arnie Heller

Key Words: blood–brain barrier (BBB), brain, brain-on-a-chip, Center for Micro and Nanotechonology, central nervous system (CNS), Forensic Science Center, heart, hippocampus, iCHIP (in vitro chip-based human investigational platform), Laboratory Directed Research and Development (LDRD) Program, microelectrodes, neurons, peripheral nervous system (PNS), Strategic Initiative (SI).

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